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APPLE-TREE RESPONSE TO NITROGEN APPLIED AT DIFFERENT SEASONS[1]

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INTRODUCTION

At present nitrogen occupies a far more important place in the fertilization of apple (*Malus sylvestris* Mill.) orchards than any of the other nutrient elements. Ballard and Volck (*2*)[3] in 1912 were among the first to demonstrate experimentally that apple trees would respond favorably to applications of nitrogen. During the following decade results of numerous investigations showed conclusively the value of nitrogen in increasing growth and yield of apple trees. So marked were the responses obtained that the use of nitrogenous fertilizers for apples spread rapidly, and at present their use in some form is almost universal in commercial orchards. This increased use of nitrogen has made possible marked reduction in the amount of cultivation in apple orchards.

Formerly readily available forms of nitrogen were usually applied to apple orchards in the spring a few weeks prior to the bloom period. During the last 10 to 15 years in many fruit sections there has been an increasing tendency toward fall applications. This trend seems to date from the work of Hooker (*11*), who found that the nitrogen and the starch content of spurs were increased in the spring and early summer by applying nitrogen the preceding fall rather than in the spring of the current year.

Since the experiments of Hooker (*11*) much work has been done relative to the best time to apply nitrogenous fertilizers in orchards (*1*, *14*, *15*, *16*, *17*). The lack of agreement in the results obtained has led to differences of opinion among horticulturists as to the most desirable season to make applications. Recommendations for the eastern part of the United States vary from early-spring to fall applications; the latter are made from early September to mid-December. Split applications are also advocated, part of the nitrogen being applied in the spring and the remainder in the fall.

In the past many of the investigations relating to season of nitrogen applications were conducted with trees that were initially in a low

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[3] Italic numbers in parentheses refer to Literature Cited, p. 25.

state of vigor, and responses were measured for only 1 or 2 years after treatment. Had such studies been of longer duration or had they been carried on with trees initially in a moderate or high level of vigor, it is possible that different conclusions might have been drawn. In commercial orchards moderately vigorous trees that receive annual applications of nitrogenous fertilizers are the rule rather than the exception. It was, therefore, the purpose of the present experiments to determine the effect on tree functions of annual applications of nitrogen made at different seasons of the year. The trees used over a period of several years were maintained at a moderately high level of vigor and in good production.

MATERIALS AND METHODS

The present studies were conducted in three rather widely separated apple orchards; two of these (the McDonald and the Dillon) were located in the Cumberland-Shenandoah fruit area and one at the Plant Industry Station, Beltsville, Md.

In the McDonald orchard near Charles Town, W. Va., 33-year-old York Imperial trees were used. These trees were growing in permanent bluegrass sod in Hagerstown clay loam. They had previously received yearly applications of nitrogenous fertilizer and were in good vigor. Hagerstown clay loam is considered an excellent soil for fruit trees. It is of residual origin, has an average field capacity of approximately 30 percent moisture, is well drained, and is of such physical make-up that it affords deep root penetration.

From the fall of 1937 through the 1941 season yearly applications of 10 pounds of $NaNO_3$ (sodium nitrate) per tree were made to five randomized trees per treatment at the following seasons:

1. Early fall (during September unless otherwise noted).
2. Late fall (November 15 to December 1, after the trees had shed their leaves).
3. Early spring (during April, approximately 3 weeks prior to bloom).
4. Late spring (during May, at the time of petal fall).
5. Midsummer (during the second half of July).

In addition, five trees received no nitrogen fertilizer after the grower's application in the spring of 1937. Fall treatments were begun in 1937 and spring and midsummer ones in 1938. Prior to treatment a sparse growth of sod beneath each tree was removed from the area of application. The nitrate of all applications was evenly distributed beneath each tree from the trunk out to approximately the outer spread of the branches. It approximated 45 p. p. m. (parts per million) of nitrate nitrogen in a foot of soil in the area covered.

In the Dillon orchard near Hancock, Md., 25-year-old Delicious trees were used. These trees were growing in a Dekalb shale loam, a shallow soil (usually not more than 2 to 2½ feet to unweathered shale) with a field capacity of approximately 20 percent moisture. Because of the limited depth of rooting on such soil fruit trees frequently lack sufficient moisture for optimum growth. Only 8 pounds of sodium nitrate per tree instead of 10 pounds was used on these smaller trees; this amount gave approximately the same concentration (45 p. p. m.) in the area to which it was applied. The experimental design was identical with that described for the York Imperial trees.

After four seasons of fertilizer treatment, two of the check trees in each orchard received nitrate in the spring of 1942 and five trees in each orchard that had received one of the spring applications were left untreated, to determine how rapidly change in the nitrogen levels of the trees would occur.

In the orchard at the Plant Industry Station, Beltsville, Md., trees 7 years old at the start of the experiment were used. They were growing in Sassafras gravelly loam of rather low fertility, maintained in a grass sod. Five trees of each of three varieties (York Imperial, Starking Delicious, and Rome Beauty) were included for each treatment. The treatments were on individual trees randomized throughout the orchard. The initial rate for a full application was 2½ pounds of sodium nitrate per tree. This amount was increased as the trees increased in size (see tables 7 and 8). The experiment included nine treatments. Five of these represented seasons of application similar to those in the McDonald and Dillon orchards. Two additional treatments consisted of nitrogen applied at the early-spring and late-fall dates, but in only half the amounts of the full treatments. Another treatment was of the full yearly amount, but split between two application dates (late-spring and early-fall); while the final treatment consisted of only half the full application, but also split between the two dates.

Since one of the chief objectives of these experiments was to determine nitrogen intake and utilization as related to season of application, samples for nitrogen analysis of blossoms, shoots, leaves, bark, wood, and roots were taken at intervals throughout the course of the experiments. These tissues were analyzed for total nitrogen by the official Kjeldahl-Gunning-Arnold method. In 2 of the orchards soil samples were taken at intervals with sampling tubes at 4 points under each tree, in order to follow the nitrate distribution at the various soil depths resulting from the different treatments. The phenoldisulfonic acid method was used, and the results were expressed in parts per million of nitrate nitrogen on the basis of air-dry soil. All sampling of soil, as well as of plant tissue, was done on an individual-tree basis. Leaf samples comprised 1 leaf midway of the shoot from each of 30 representative terminal shoots. Dormant and current shoots, as well as blossom clusters at appropriate sampling dates, were taken at 30 to 50 points throughout the tree. Root samples were taken in the surface foot of soil at 4 places beneath the spread of the branches. Unless otherwise stated, only the small roots less than about 6 mm. in diameter were selected for analysis.

Fruit-set records were obtained by counting 1,200 to 2,000 blossom clusters on 4 typical limbs in each tree. Two of these limbs were near the top of the tree and 2 in the lower half. Fruit set on these limbs was determined after the June drop. Color of fruit was determined at harvest by separating all of the fruit into 4 color grades based on the percentage of the total surface of good red color. The total average color for each tree was then calculated from the amount of fruit falling into each grade. Each year careful growth and bloom estimates were made on an individual-tree basis, and yield and fruit-size records were obtained. In the young orchard at Beltsville increase in trunk circumference was used as a measure of growth.

EXPERIMENTAL RESULTS IN McDONALD AND DILLON ORCHARDS

NITRATE-NITROGEN CONTENT OF SOIL

McDONALD ORCHARD

Root absorption, leaching, and biological fixation are the most common factors responsible for changes in the nitrate-nitrogen content of the soil after applications of sodium nitrate. Data for the nitrate nitrogen recovered at various depths in the McDonald orchard after the early-fall, the late-fall, the early-spring, the late-spring, and the midsummer applications are shown in figures 1 to 5, respectively.

As stated previously, the applications of sodium nitrate were calculated to give approximately 45 p. p. m. of nitrate nitrogen in a 1-foot depth of soil. From the data in figure 1 it is apparent that after early-fall applications most of the nitrate applied was recoverable even in the following early spring. Thus, almost as much nitrate was recovered on April 20, 1938, as had been applied on October 15, 1937. In 1939 the spring sampling was not made until May 11, approximately 2 weeks after full bloom. By that time the recoverable nitrate was approximately half the amount applied on September 2, 1938. Similarly, of the amount applied on September 2, 1939, approximately three-fourths was recovered on April 2, 1940, before the beginning of growth. The only exception to a relatively high recovery in the early spring was in 1941, when only approximately one-fourth as much nitrate was recovered on April 9 as had been applied the previous September. Rainfall had been unusually heavy after the date of application in the fall of 1940.

From October 15, 1937, to April 20, 1938, approximately 15 inches of rain fell. Most of the nitrate recovered on the later date was in

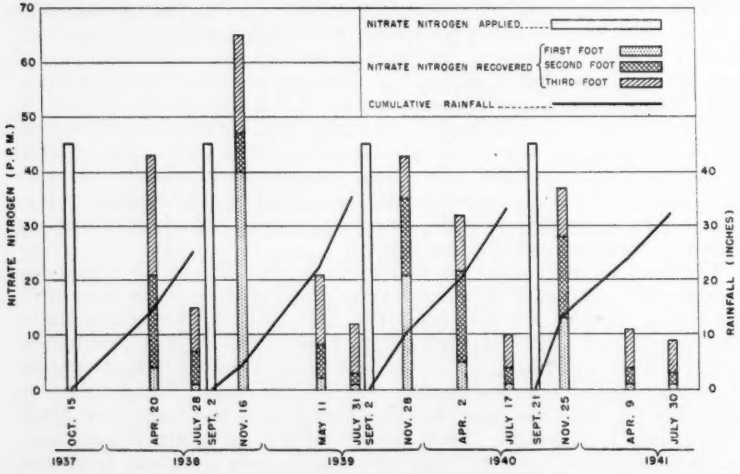

FIGURE 1.—Nitrate nitrogen applied annually in early fall and amounts recovered from the soil at various intervals thereafter, McDonald Orchard. Cumulative rainfall from date of application also indicated.

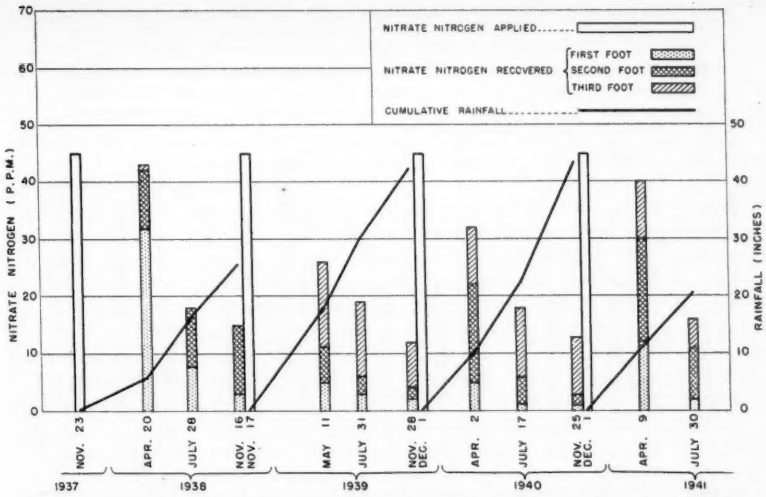

FIGURE 2.—Nitrate nitrogen applied annually in late fall and amounts recovered from the soil at various intervals thereafter, McDonald orchard. Cumulative rainfall from date of application also indicated.

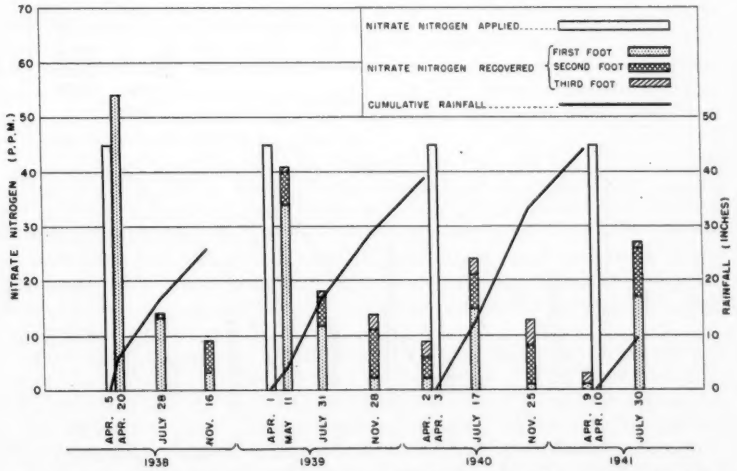

FIGURE 3.—Nitrate nitrogen applied annually in early spring and amounts recovered from the soil at various intervals thereafter, McDonald orchard. Cumulative rainfall from date of application also indicated.

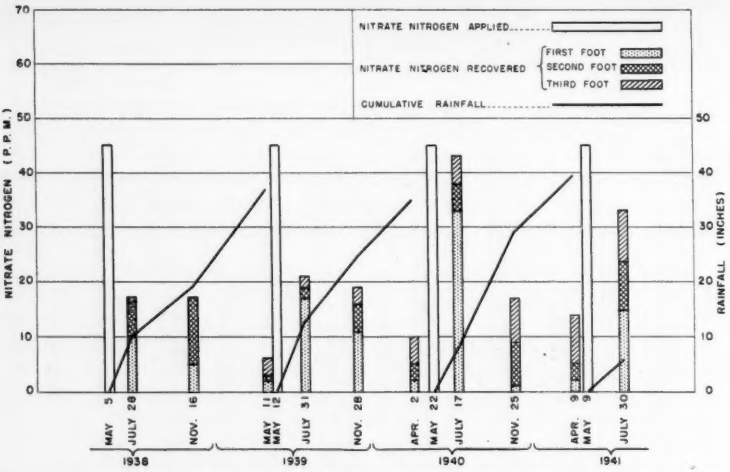

FIGURE 4.—Nitrate nitrogen applied annually in late spring and amounts recovered from the soil at various intervals thereafter, McDonald orchard. Cumulative rainfall from date of application also indicated.

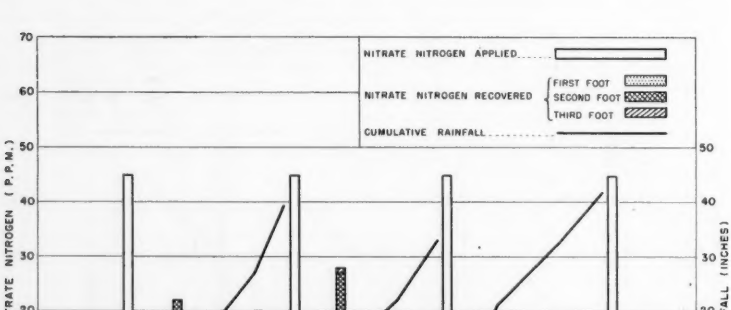

FIGURE 5.—Nitrate nitrogen applied annually in midsummer and amounts recovered from the soil at various intervals thereafter, McDonald orchard. Cumulative rainfall from date of application also indicated.

the second and third feet of soil. Between September 2, 1938, and May 11, 1939, there were 22.5 inches of rain. Most of the nitrate recovered on the later date was in the third foot of soil. From September 2, 1939, to April 2, 1940, 20 inches of rain fell, whereas from September 21, 1940, to April 9, 1941, the rainfall was just over 24 inches. It is possible that the heavier rainfall during the fall and winter of 1940–41 resulted in appreciable leaching of nitrate to below the 3-foot depth of sampling.

It is of interest to note that, although most of the nitrate that had been applied in the fall could be recovered in the following early spring, under the conditions of this test most of it was in the second- and third-foot depths. Relatively small quantities remained in the surface foot, where tree-root concentration is highest. However, in this orchard there was fairly good root distribution to a depth of at least 4 to 5 feet.

In general the pattern for nitrate recovery from late November applications was very similar to that from September applications (fig. 2). Most of the nitrate could be recovered the following spring and most of it was in the second and third feet of soil. The only exception occurred in the spring of 1938 when, after a very dry winter with only 6 inches of rainfall between November 23, 1937, and April 20, 1938, most of the nitrate was still in the top foot.

Figure 3 shows the nitrate recovery from early-spring applications. Shortly after the spring application, practically all of the nitrate could be recovered. By midsummer, represented by the late July samplings, on the average about half of the nitrate had disappeared. It is of interest to note, however, that even at the time of leaf fall in November, approximately one-fourth of the nitrate that had been applied in the spring could still be recovered. It seems probable that most of this disappearance of nitrate nitrogen was by root absorption. In these experiments there was little evidence of disappearance of nitrate nitrogen at seasons of the year when the trees were not in an active state of growth.

It is also of interest to note that at the time of the midsummer sampling (late July) most of the recoverable nitrate from early-spring applications was still in the top foot of soil even though 10 to 17 inches of rainfall had occurred. Apparently the trees dried out the soil between periods of rainfall, and there was relatively little movement of nitrate from the top foot into the lower soil depths under these conditions. Some was recovered in the second foot, but almost none in the third foot.

After the late-spring applications (May) there were relatively larger amounts of nitrate retained in the soil in July and in November than after the early-spring applications (fig. 4). There appeared to be relatively little disappearance between July and November. This relatively limited disappearance during late summer was evident also from the early-spring applications. If it is assumed that the disappearance was largely a result of absorption by the trees, the results would indicate that the first half of the growing season, or the period of rapid leaf expansion, was the period of most rapid absorption of nitrates from the soil.

When the fertilizer was applied in late July, approximately half of the nitrate had disappeared by late November (fig. 5). Thus, ab-

sorption during the late summer appeared to be relatively more active on trees that had not received applications in the spring. These trees were relatively low in nitrogen at the time of application, which was after terminal and leaf growth had been completed for the season. Under these conditions absorption appeared to be relatively greater during the late summer than in trees better supplied with nitrate as a result of the spring application.

Dillon Orchard

Because the Dillon orchard is growing on relatively shallow soil with undecomposed shale at a depth of 2 to 2½ feet, it was not possible to sample the soil below the 2-foot depth. The soil was somewhat lighter in texture with less water-holding capacity than that of the McDonald orchard. Since much of the nitrate applied in the McDonald orchard was carried below the 2-foot depth by the following spring, it may be presumed that much of the nitrate in the Dillon orchard was carried to a depth below that of possible sampling. Rainfall data were not available from any point near the Dillon orchard, but it may be presumed that the seasonal averages were approximately similar to those at the McDonald orchard, about 35 miles away.

The data in figures 6 to 10 for the various dates of application of nitrate nitrogen at the Dillon orchard bear out these probabilities. After early-fall applications generally less than one-fourth of the nitrate could be recovered the following spring. Much of that not recoverable may be presumed to have leached below the depth of possible sampling and possibly below the rooting depth. Data on nitrogen content of the roots presented later (table 1), however, indicate that during the dormant season some intake of nitrogen occurred in these trees.

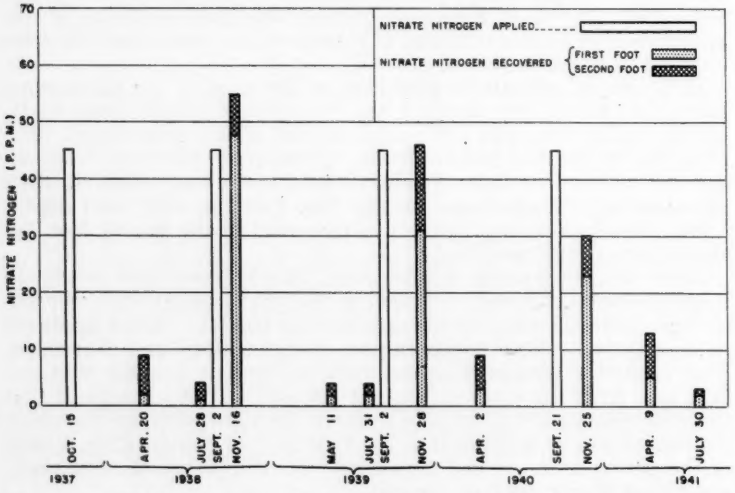

Figure 6.—Nitrate nitrogen applied annually in early fall and amounts recovered from soil at various intervals thereafter, Dillon orchard.

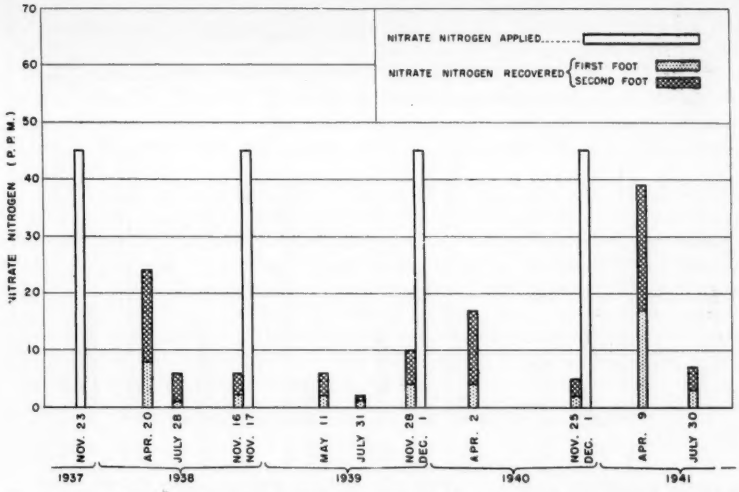

FIGURE 7.—Nitrate nitrogen applied annually in late fall and amounts recovered from soil at various intervals thereafter, Dillon orchard.

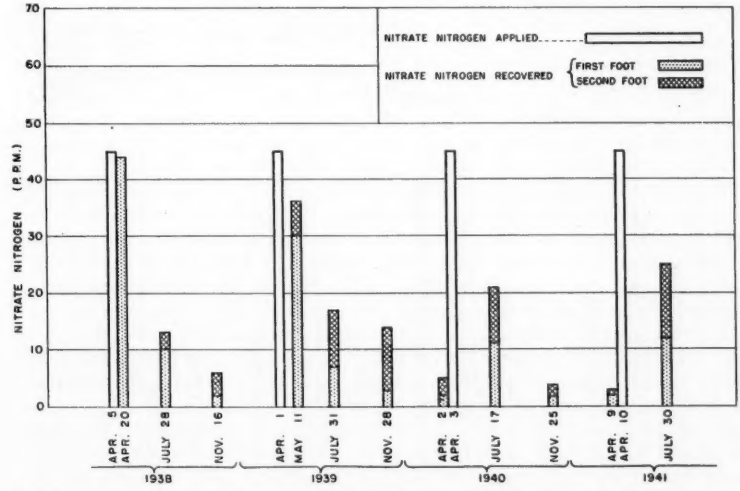

FIGURE 8.—Nitrate nitrogen applied annually in early spring and amounts recovered from soil at various intervals thereafter, Dillon orchard.

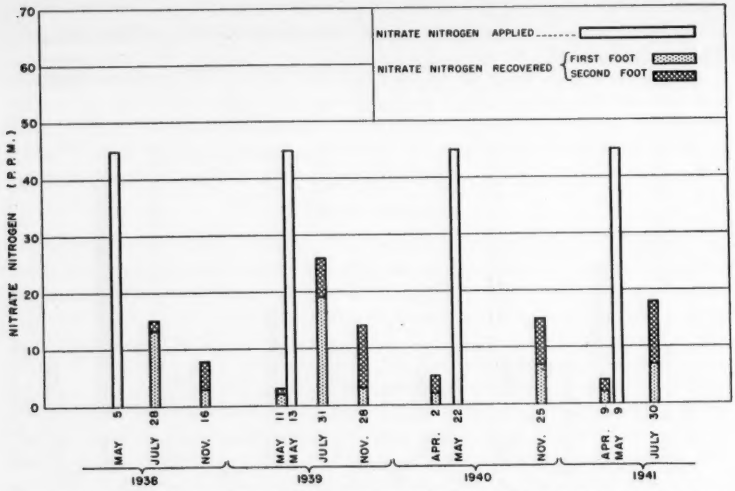

FIGURE 9.—Nitrate nitrogen applied annually in late spring and amounts recovered from soil at various intervals thereafter, Dillon orchard.

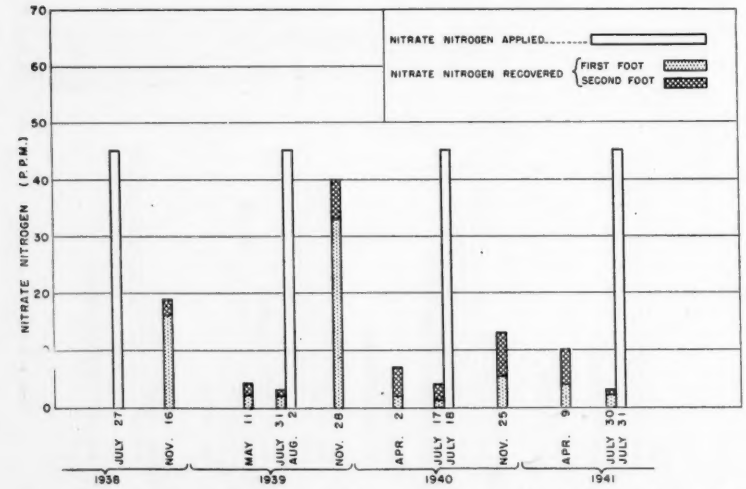

FIGURE 10.—Nitrate nitrogen applied annually in midsummer and amounts recovered from soil at various intervals thereafter, Dillon orchard.

For the early-spring applications the pattern is substantially similar to that of the McDonald orchard. On the average, approximately one-half of the nitrate could be recovered in late July and there was a rather marked further decrease between July and the end of the growing season. The results from the late-spring and midsummer applications also were substantially similar to those for the McDonald orchard.

Although there was little evidence of marked absorption of nitrate during the dormant season, the results on disappearance of nitrate in these two orchards would indicate that on a deep and fairly retentive soil with good root distribution there is little probability of loss of nitrates to the deep subsoil below the zone of root penetration when the nitrate is applied in the fall. On the other hand, where the depth of rooting is restricted by hardpan or rock or for other reasons, considerable of the nitrate applied in the fall may be carried below the active root zone before the following spring.

NITROGEN CONTENT OF VARIOUS PARTS OF TREES

Roots

Several investigators (*1*, *4*, *16*, *17*) have reported the absorption of nitrogen by fruit-tree roots during the winter months when the tree tops are dormant. The data on Delicious apples (table 1) indicate significant increases in the nitrogen content of the roots during the dormant period that followed applications of nitrogen in the fall of 1937. Also in this experiment, during the dormant period of 1938–39 the nitrogen treatments in which the nitrogen content of roots was at a relatively low level at the beginning of the dormant period (early-spring, late-spring, and late-fall treatments) again showed appreciable increases prior to the beginning of growth the following spring. During this same period the midsummer and early-fall treatments, with a higher nitrogen content on December 1, 1938, showed no appreciable change in root nitrogen from that sampling date to March 15, 1939. The authors (*4*) previously showed that roots with a lower nitrogen content at the beginning of dormancy had a much greater rate of increase in nitrogen intake during the dormant period; the lower rate of increase in roots with the higher nitrogen content was attributed to the fact that these roots were nearer to a condition of nitrogen equilibrium when the tree entered the dormant period.

As evidenced by the data in table 1, only a small increase in nitrogen content of the roots of York Imperial trees took place during dormancy and the increase was as great in the check trees as in those receiving nitrate. In view of the recent work of Hoagland and Arnon (*10*), showing a close relation of oxygen supply of the rooting medium to nutrient absorption, the possibility of insufficient aeration in this heavier textured soil may have been a contributing factor. The work of the authors (*4*), which showed the nitrogen increase in the roots of dormant apple trees growing in pots in sandy loam soil to be more than double that of trees similarly grown in clay loam, would seem to lend support to this hypothesis.

The average nitrogen content of the roots for the various treatments immediately before the initiation of growth are presented in the last column of table 1. It may be seen that the early-spring application

TABLE 1.—*Relation of nitrogen content of roots to season of nitrogen application, 1937–41*

Variety and treatment	Nitrogen content [1] (dry-weight basis)						
	Nov. 20, 1937	Mar. 17, 1938	Dec. 1, 1938	Mar. 15, 1939	Apr. 2, 1940	Apr. 8, 1941	Average (March and April)
York Imperial (McDonald orchard):	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Check	0.61	0.69	0.49	0.61	0.56	0.52	0.59
Early-fall	.61	.69	.65	.72	.73	.65	.70
Late-fall	.53	.68	.60	.71	.66	.79	.71
Early-spring			.54	.64	.62	.75	.67
Late-spring			.65	.70	.79	.63	.71
Midsummer			.74	.84	.86	.78	.83
Delicious (Dillon orchard):							
Check	.69	.84	.52	.57	.55	.56	.63
Early-fall	.85	1.13	1.13	1.17	1.26	1.18	1.18
Late-fall	.70	1.33	1.02	1.18	1.19	1.06	1.19
Early-spring			.80	1.05	.81	.81	.89
Late-spring			.95	1.14	.93	1.18	1.08
Midsummer			1.24	1.20	1.21	1.16	1.19

[1] Difference necessary for significance at 5-percent point: 0.19.

to the Delicious trees resulted in a significantly lower nitrogen content of roots at that time than did the other nitrogen treatments; among these others there was no significant difference. The nitrogen content of roots of check trees, to which no nitrogen was applied throughout the course of the experiments, was significantly lower than that of those given the various treatments. In the York Imperial experiment, however, the roots of check trees did not contain significantly less nitrogen at the time the samples were taken than did the roots of trees receiving nitrogen except in the midsummer application; nevertheless, they always averaged considerably lower.

The data on root analysis as a whole indicate some increase in nitrogen content of the roots during the dormant season, particularly of those of the Delicious on well-aerated soil. They do, however, corroborate the results of the study of the nitrates in the soil, which indicated only a limited absorption of nitrogen in the McDonald orchard during the dormant season by trees previously well supplied with nitrogen.

DORMANT TERMINAL GROWTH

The data presented in table 2 show no consistent changes in nitrogen content of shoots during dormancy (comparison of November or December and March analyses). In both experiments the nitrogen level of dormant shoots of the fertilized trees averaged higher than that of shoots of the unfertilized trees. However, there was little or no difference in nitrogen content of shoots from the different treatments, except for those of the Delicious trees that received the midsummer application. The content of shoots of the Delicious that received this treatment was in general significantly higher than that of the shoots that received the other treatments.

BLOSSOMS

During 1938, 1939, and 1941 samples of blossoms were taken at the most advanced "balloon" stage, just before opening, to determine whether there were differences in the nitrogen content due to nitrogen

TABLE 2.—*Relation of nitrogen content of dormant terminal shoots to season of nitrogen application, 1937–41*

Variety and treatment	Nitrogen content [1] (dry-weight basis)						
	Nov. 20, 1937	Mar. 17, 1938	Dec. 1, 1938	Mar. 15, 1939	Apr. 2, 1940	Apr. 8, 1941	Average (March and April)
York Imperial (McDonald orchard):	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Check	0.91	0.95	0.91	0.90	0.90	0.87	0.90
Early-fall	.98	1.02	1.08	.99	1.03	1.02	1.01
Late-fall	.92	.98	1.00	1.02	.95	1.02	.99
Early-spring			1.03	.93	.98	.97	.96
Late-spring			1.07	1.05	1.05	.99	1.03
Midsummer			1.12	1.04	1.09	.99	1.04
Delicious (Dillon orchard):							
Check	.85	.91	.93	.86	.90	.96	.91
Early-fall	.94	.96	1.10	1.00	1.05	1.07	1.02
Late-fall	.95	1.02	1.08	1.05	1.01	1.05	1.03
Early-spring			1.06	1.05	1.05	.99	1.03
Late-spring			1.11	1.00	1.05	1.05	1.03
Midsummer			1.08	1.14	1.12	1.20	1.15

[1] Difference necessary for significance at 5-percent point: 0.10.

treatment (table 3). There was little difference in the York Imperial blossoms resulting from the treatments, the blossoms from the check trees being about as high in nitrogen as those from the trees receiving the various treatments. The midsummer application resulted in the highest nitrogen level in the blossoms. The Delicious blossoms from the check trees were generally lower in nitrogen than those from fertilized trees, the differences being generally significant in 1938 and 1939, but not in 1941. The nitrogen content was generally above 4 percent of the dry weight in the case of the Delicious and a little lower in the case of the York Imperial. Thus, at the balloon stage approximately one-fourth of the dry material of the blossoms is of nitrogenous compounds, since proteins average about 16 percent nitrogen.

TABLE 3.—*Relation of nitrogen content of blossoms and of current shoot growth to season of nitrogen application, 1938–41*

Variety and treatment	Nitrogen content [1] (dry-weight basis)							
	1938					1939		1941
	Current shoot growth				Blossoms (Apr. 19)	Current shoot growth (Apr. 25)	Blossoms (Apr. 25)	Blossoms (Apr. 25)
	Apr. 19	Apr. 26	May 4	May 23				
York Imperial (McDonald orchard):	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Check	3.75	2.99	2.52	1.93	3.61	3.91	3.44	3.10
Early-fall	3.77	3.13	2.76	2.04	3.71	3.95	3.46	3.11
Late-fall	3.82	3.12	2.53	2.00	3.92	3.92	3.48	3.15
Early-spring	3.82	3.19	2.66	2.02	3.80	3.94	3.44	3.16
Late-spring				2.07		3.96	3.41	3.10
Midsummer						3.91	3.71	3.24
Delicious (Dillon orchard):								
Check	4.25	3.66	2.95	2.48	3.92	4.85	4.16	3.59
Early-fall	4.58	4.04	3.53	2.76	4.12	5.10	4.41	3.73
Late-fall	4.58	4.07	3.55	2.73	4.26	5.02	4.36	3.69
Early-spring	4.47	3.94	3.34	2.89	4.16	5.03	4.40	3.65
Late-spring				2.72		5.02	4.37	3.55
Midsummer						5.13	4.33	3.70

[1] Difference necessary for significance at 5-percent point: Shoot growth, 0.06; blossoms, 0.20.

There were marked differences in the nitrogen content of the blossoms in the different seasons. In 1941 the nitrogen content of the blossoms in every treatment was markedly below the corresponding 1939 levels. In the spring of 1939 there were no unusually warm days prior to the date of sampling, April 25. Of the previous 10 days, only 3 had had maximum temperatures as high as 70° F. Throughout late March and April, however, temperatures were sufficiently high to permit a rather steady development of the blossoms. In March 1941, on the other hand, practically no days were sufficiently warm to cause growth and little growth occurred until after the first week in April. Beginning with April 11, the maximum temperatures were above 70° every day through April 21, and on eight of these days they were above 80°. Thus, the blossoms and new shoot growth developed with extreme rapidity during the 2 weeks prior to sampling. It seems probable that this very rapid development was mainly responsible for the lower nitrogen content in the succulent blossom tissues. The translocation of nitrogen may have been relatively less affected by the higher temperatures than was the growth rate.

The number of fruit set per 100 blossoming spurs of both the Delicious and the York Imperial was actually higher in 1941, when the nitrogen content was low, than in 1939, when the nitrogen content was much higher. Some low-temperature injury had occurred prior to blossoming in 1939, but it did not appear to be extensive. In both years conditions for pollination were very favorable. Thus, under the conditions of this test the lower nitrogen content of the blossoms was not unfavorable for fruit set. Furthermore, throughout the full course of the experiment the fruit set on the check trees averaged as high as that on the nitrogen-treated trees (see table 6).

Current Shoot Growth

In the spring of 1938 current shoot growth was sampled four times during terminal elongation (table 3). On April 19, soon after the beginning of growth, the shoots (about half an inch long) on check trees had a slightly lower nitrogen content in most cases than those on trees treated at various seasons. The content of the shoots on the Delicious trees treated in early spring was lower than that of those treated at other seasons; otherwise season of treatment apparently had little effect. At the time of terminal-bud formation (about May 23), however, shoots from the Delicious trees treated in early spring had the highest nitrogen content. On this date and also at blossoming time in 1939 shoots from York Imperial trees that had received various treatments showed only slight differences, although all fertilized trees were significantly higher than the checks. In 1939 shoots from treated Delicious trees all had a higher nitrogen content than those from check trees, and those treated in midsummer and early fall had a significantly higher content than those fertilized at other seasons.

The data on current shoot growth shown in table 3 indicate also the rapid absorption and translocation of nitrogen resulting from spring applications. In 1938, after nitrogen applications on April 5 (early spring) and May 6 (late spring), the nitrogen content of current shoot growth of both varieties showed a significant increase when compared with check trees within 14 and 17 days, respectively. The

nature of the experiment made it impossible to check this result in other years.

The shoots all showed a rapid decline in nitrogen from the initiation of new growth until the end of the sampling period. On the average terminal growth ceased somewhat earlier on the check trees than on the fertilized ones. All trees had ample moisture when shoot elongation ceased. These data indicate that the time of cessation of terminal growth in mature apple trees is largely determined by the depletion of the nitrogen available for rapid transfer to the new developing tissues—assuming no other nutrient element to be limiting. The amount of the new terminal growth appears to be determined by the reserve nitrogen in the tree and the intake during the season of rapid development of new tissues.

Foliage

Numerous investigators (*3*, *7*, *8*) have shown the nitrogen level of the leaves to be a very sensitive index to the nitrogen supply in the rooting medium and have generally reported a very close relation between the nitrogen content of foliage and the growth and vigor of fruit trees. The close relation between nitrogen content of foliage and leaf assimilation per unit area has also been established (*3*, *9*).

The nitrogen content of leaves taken from the median parts of terminal shoots at intervals throughout the experiment is shown in table 4. In many instances differences statistically significant were obtained between certain treatments in a particular year, whereas in another year at approximately the same date there seemed to be either no difference or the difference was even in the opposite direction in certain cases.

Throughout the experiment there was no consistently significant difference between the nitrogen content of the foliage after early-fall, late-fall, or early-spring treatments. During the growing season the foliage from check trees was consistently lower in nitrogen than that from trees receiving nitrogen applications.

It might be expected that the late-spring application, made after most of the leaf system was expanded and too late to influence greatly the growth for the season, would result in a higher level of nitrogen in the foliage throughout the summer than would the dormant-season treatment. This was generally true of the York Imperial but not of the Delicious. The over-all average in June of nitrogen in the foliage of the Delicious variety resulting from the late-spring application was below that resulting from application in the fall or early spring. No explanation of these results is apparent.

It would be expected that for samples taken in early summer the midsummer applications would result in a somewhat lower nitrogen content of the leaves than would dormant-season or late-spring applications. This was true of the York Imperial, as shown by the average for the June samples. It was also true of the Delicious except that there was no difference between the late-spring and midsummer appplications.

During September the nitrogen content of the foliage of all trees that received nitrogen was essentially the same irrespective of the season of application (table 4). Heinicke (*9*) emphasized the importance of making a late-summer application of nitrogen in order to

TABLE 4.—*Relation of nitrogen content of foliage to season of nitrogen application, 1938–41*

Variety and treatment	Nitrogen content [1] of terminal leaves (dry-weight basis)															
	1938					1939			1940		1941		Average	
	May 24	June 22	Aug. 1	Sept. 13	Nov. 2	June 15	Aug. 1	Sept. 1	June 11	Sept. 19	June 17	Sept. 10	June	Sept.
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
York Imperial (McDonald orchard):														
Check	1.99	2.08	1.93	1.78	0.89	1.79	1.81	1.81	2.02	1.65	1.94	1.64	1.96	1.72
Early-fall	2.21	2.43	2.17	1.95	.92	2.12	2.12	2.04	2.17	1.82	2.27	1.92	2.25	1.93
Late-fall	2.14	2.37	2.13	1.94	.89	2.13	2.09	2.06	2.29	1.83	2.30	1.93	2.27	1.94
Early-spring	2.10	2.35	2.19	1.97	.87	2.17	2.14	2.05	2.28	1.87	2.27	1.94	2.27	1.96
Late-spring	2.17	2.46	2.23	2.03	.95	2.18	2.15	2.01	2.48	1.88	2.31	1.80	2.36	1.93
Midsummer	----	----	2.04	2.01	.90	2.15	2.11	2.06	2.18	1.91	2.20	1.86	2.18	1.96
Delicious (Dillon orchard):														
Check	1.99	2.07	1.87	1.62	1.21	1.81	1.78	1.65	1.82	1.68	2.14	1.88	1.96	1.71
Early-fall	2.33	2.39	2.13	1.89	1.18	2.48	2.22	2.03	2.46	2.10	2.42	2.03	2.44	2.01
Late-fall	2.27	2.47	2.19	1.95	1.17	2.45	2.23	2.03	2.43	2.06	2.44	2.07	2.45	2.03
Early-spring	2.38	2.52	2.16	1.94	1.14	2.40	2.23	2.09	2.49	2.14	2.47	2.13	2.47	2.07
Late-spring	2.27	2.43	2.22	1.98	1.10	2.25	2.22	2.12	2.49	2.10	2.31	1.98	2.37	2.04
Midsummer	----	----	1.93	1.83	1.12	2.28	2.10	2.02	2.43	2.20	2.37	2.11	2.36	2.04

[1] Difference necessary for significance at 5-percent point: 0.05.

maintain or increase the dark-green color of apple foliage and so increase its efficiency in assimilation during the late summer and fall. With nitrogen-deficient trees such a practice would probably be beneficial and would doubtless accomplish the desired purpose. However, with trees that received annual application of nitrogenous fertilizers no difference was found in nitrogen content of foliage or in leaf color during the fall months as a result of differences in season of application. Since the close relation between nitrogen content and color and efficiency of foliage has been demonstrated (*3*, *6*), there is little likelihood that any real difference in foliage assimilation that could be attributed to season of application occurred during late summer and fall in these experiments.

In 1938 the leaves were sampled at rather frequent intervals from late May until leaf fall. It is particularly interesting to note that samples of both varieties taken May 24 were consistently lower in nitrogen than those taken June 22. These results suggest that the leaves reach a low level of nitrogen content in the spring at about the time of cessation of terminal growth and that subsequently there is some increase in nitrogen in the foliage. Samples taken in midsummer (August 1) were generally slightly lower in nitrogen than those taken in June. The trend of nitrogen content was downward from June until September.

One set of analyses, the November 2 sampling, were made on leaves that fell when the branches were jarred. Approximately half of the foliage had dropped when the samples were collected. Branches were jarred and leaves that fell were taken and analyzed. In the York Imperial the nitrogen content of leaves was less than half the midsummer level, whereas in the Delicious it was slightly more than half. Thus, although much of the nitrogen apparently moved out of the leaves prior to leaf fall, a substantial portion was still present at that time.

In general, the leaf analyses indicate no significant differences in the levels of nitrogen maintained in these trees as a result of varying the time of application of sodium nitrate. Even in the Delicious, for which the data on nitrate in the soil indicated some loss of nitrates from leaching after the fall applications, this loss was not reflected in the nitrogen level maintained in the leaves. The nitrogen level resulting from the fall applications was as high as that from spring and midsummer applications.

Various Tissues of Dormant Trees After 4 Years of Treatment

Much of the nitrogen contained in the various tissues of the tree is available for retransfer and reutilization. After 4 years of treatment it seemed desirable to determine to what extent the amount of reserve nitrogen contained in the different parts of the tree had been influenced by season of nitrogen application. Trees receiving the late-fall and early-spring treatments were selected for detailed analysis, since in many fruit districts it is at these seasons that nitrogenous fertilizers are most frequently applied.

The data for all the various tissues analyzed for nitrogen content do not show any differences of significance attributable to season of application (table 5). With some tissues certain numerical differences are apparent, but such differences are not consistent enough or of sufficient magnitude to be definitely related to season of application.

It will be noted from the data in table 5 that the various tissues of the unfertilized trees generally contain less nitrogen than those of the trees receiving nitrogen, the differences being greatest in the bark of limbs and the root tissues. By careful analyses Magness and Regeimbal (*13*) estimated a necessary yearly intake of about 1.5 pounds of nitrogen to maintain a mature apple tree in good production. From the nitrogen values contained in table 5 and the dry weights of the

TABLE 5.—*Relation of nitrogen content of various tissues of dormant trees after 4 years of treatment to season of nitrogen application, December 1, 1941*

Variety and treatment	Nitrogen content (dry-weight basis)												
	Tops										Roots		
	Small limbs [1]			Medium limbs [2]			Large limbs [3]				Less than 5-mm. diameter	Large roots	
	Shoots	Bark	Wood	Bark	1-year-old sapwood	Old sapwood	Bark	1-year-old sapwood	Old sapwood	Heartwood		Bark	Wood
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
York Imperial (McDonald orchard):													
Check	0.937	0.830	0.245	0.609	0.348	0.134	0.623	0.302	0.118	0.087	0.562	0.427	0.187
Late-fall	1.028	.963	.301	.650	.375	.134	.684	.328	.121	.090	.579	.418	.300
Early-spring	1.039	.941	.258	.665	.355	.131	.688	.291	.121	.088	.638	.488	.251
Delicious (Dillon orchard):													
Check	.912	.881	.246	.700	.260	.136	.671	.251	.137	.102	.585	.459	.342
Late-fall	1.110	.897	.250	.743	.289	.150	.720	.292	.146	.103	1.024	.608	.416
Early-spring	1.133	.950	.273	.754	.256	.144	.764	.260	.146	.109	.958	.587	.537
Difference necessary for significance at 5-percent point	.116	.077	.044	.043	.044	.012	.054	.044	.008	.011	.241	.160	.204

[1] ½- to ¾-inch diameter. [2] 1- to 2-inch diameter. [3] Over 3-inch diameter.

various tissues of an apple tree, it was estimated that the nitrogen-fertilized trees contained 0.6 to 0.7 of a pound more residual, or reserve, nitrogen than the unfertilized ones. This amount of nitrogen, which might be regarded as available for retransfer and reutilization, would be sufficient to meet a substantial proportion of the requirement of a tree for 1 year.

SET, COLOR, AND YIELD OF FRUIT

The data presented in table 6 show considerable consistency in fruit set of trees treated at different seasons. Average fruit set for the 4-year period was essentially the same regardless of season of fertilization or lack of fertilization. The relation of fruit color to nitrogen supply and intake has been shown previously (*5*, *12*). It is interesting to note that in the present experiments little if any differences in fruit color were associated with season of nitrogen application. The much higher color of fruit from trees not receiving nitrogen is clearly shown in table 6. Although yield records on such a limited number of trees

TABLE 6.—*Relation of fruit set, color, and yield to season of nitrogen application, 1938–41*

Variety and treatment	Fruits per 100 blossoming spurs	Color	Yield	Variety and treatment	Fruits per 100 blossoming spurs	Color	Yield
York Imperial (McDonald orchard):	*Number*	*Percent*	*Bushels*	Delicious (Dillon orchard)—Continued	*Number*	*Percent*	*Bushels*
Check	44	58	19.6	Early-fall	35	49	10.6
Early-fall	39	45	19.4	Late-fall	36	49	11.6
Late-fall	41	41	19.2	Early-spring	30	47	10.8
Early-spring	44	42	21.4	Late-spring	35	50	12.0
Late-spring	37	43	20.4	Midsummer	31	53	10.6
Midsummer	40	42	21.6	Difference necessary for significance at 5-percent point	9	5	4.
Delicious (Dillon orchard):							
Check	32	66	8.0				

(five per treatment) cannot be regarded as very reliable, the data do not suggest any great differences in fruiting performance. The amount of bloom per tree and the percentage set were recorded each year and careful growth estimates (terminal growth and density and size of foliage) were made at intervals throughout each growing season. No significant differences that could be attributed to season of nitrogen application were ever observed.

Withholding nitrogen from the check trees resulted in reduced growth and relatively small, light-green leaves the first season of these experiments (1938), and these manifestations became progressively more pronounced in 1939, with the trees exhibiting typical symptoms of nitrogen deficiency during that year. The growth of these trees, however, was not greatly different in 1940 and 1941 from that in 1939. The lower average yield of the check trees in the Delicious experiment reflects this reduced growth, and in the York Imperial experiment it was obvious that the yield of the check trees would ultimately be reduced if nitrogen applications were not resumed. Of interest is the fact that throughout these experiments nitrogen never became sufficiently limited to reduce fruit set of the check trees, though these trees quickly reflected the lack of adequate nitrogen for normal vigor and growth.

RATE OF RESPONSE TO NITROGEN FERTILIZER

In the spring of 1942 nitrogen was applied to two check trees and withheld from five trees formerly receiving spring applications in each of the two experiments. In September of that year in the York Imperial experiment the foliage of the two former check trees that received nitrogen in the spring contained 1.99 percent of nitrogen, whereas the foliage of the three remaining checks that did not receive nitrogen contained 1.64 percent. The average fruit color on the nonfertilized trees was much superior to that on the fertilized ones. When nitrogen was withheld in 1942 from five of the trees formerly receiving spring applications, the nitrogen content of the foliage was 1.71 percent in September, while that of the regularly treated trees was 1.96 percent. Fruit color averaged 50 percent and 41 percent, respectively. When the same procedure was followed in the Delicious experiment in 1942, the results were very similar. Thus, in these experiments, when nitrogen was applied in early spring to trees in moderately low vigor, its effect was clearly reflected in nitrogen content of foliage and in fruit color during the fall of the same year. Also, withholding nitrogen from trees in high vigor resulted in a sharp decrease in nitrogen content of foliage and in improved fruit color within a single season.

EXPERIMENTAL RESULTS IN THE PLANT INDUSTRY STATION ORCHARD

NITROGEN CONTENT OF FOLIAGE AND TREE GROWTH

The results pertaining to season of nitrogen application in the McDonald and Dillon orchards were obtained with mature apple trees. In order to determine the response of young trees to season of application, experiments were conducted over a 5-year period (1939–43) with Rome Beauty, Starking Delicious, and York Imperial trees between their seventh and eleventh years of age. Details of the experimental set-up are presented under Materials and Methods.

That various treatments resulted in considerable difference in growth (as measured by percentage of increase in trunk circumference) and in nitrogen content of foliage is shown in table 7. As would be expected, trees receiving half the full application of nitrogen (Nos. 7, 8, 9) contained less nitrogen in the foliage during late summer and also made a lower rate of growth than those receiving high-nitrogen treatments. The three treatments in which the trees had the highest nitrogen content of foliage and the greatest trunk growth were those that received the late-spring, midsummer, and split (spring and fall) treatments in the high-nitrogen series. No significant differences in growth rate or nitrogen content of foliage resulted from early-spring, early-fall, and late-fall applications at the high level or from late-fall and early-spring applications at the low level. The close relation between nitrogen content of foliage and trunk growth of trees shown in table 7 is graphically illustrated in figure 11.

NITROGEN CONTENT OF FOLIAGE AND FRUIT COLOR

The relation of nitrogen content of foliage and fruit color of Rome Beauty trees to season of nitrogen application is shown in table 8. In the high-nitrogen series development of fruit color and nitrogen

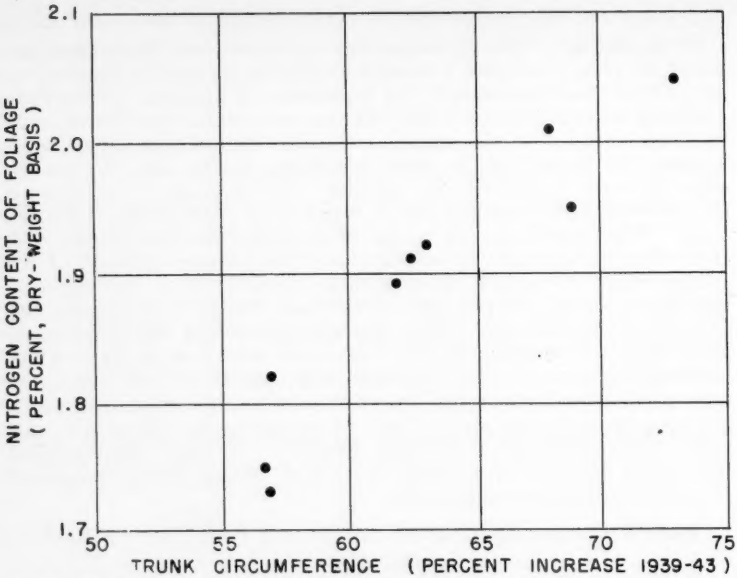

FIGURE 11.—Relation of average nitrogen content of foliage and percentage of increase in trunk circumference of York Imperial, Starking Delicious, and Rome Beauty apple trees 7 to 11 years of age.

content of foliage were essentially the same when treatments were made in early spring, early fall, and late fall; in the low-nitrogen series they were about the same when the treatments were made in early spring and late fall. With respect to the other treatments the data generally show that the treatments resulting in the highest nitrogen level of foliage and the greatest percentage of increase in growth (table 7) produced fruit with the least red color (treatments 2 and 3).

TABLE 7.—*Relation of nitrogen content of foliage and tree growth of York Imperial, Starking Delicious, and Rome Beauty apple trees to season of nitrogen application, 1939–43*

Relative rate of nitrogen application and treatment No. and description	Average increase in trunk circumference	Average nitrogen content of foliage [1]	Relative rate of nitrogen application and treatment No. and description	Average increase in trunk circumference	Average nitrogen content of foliage [1]
High: [2]	*Percent*	*Percent*	Low: [3]	*Percent*	*Percent*
1. Early-spring	62.5	1.91	7. Early-spring	56.9	1.73
2. Late-spring	73.0	2.05	8. Late-fall	56.8	1.75
3. Midsummer	68.9	1.95	9. Split (half late-spring, half early-fall)	56.9	1.82
4. Early-fall	63.3	1.92	Difference necessary for significance at 5-percent point	6.6	.05
5. Late-fall	61.9	1.89			
6. Split (half late-spring, half early-fall)	67.9	2.01			

[1] Leaves from terminal growth taken for analysis in late July or early August.
[2] Yearly applications of actual nitrogen (pounds per tree) were as follows: 1939, 0.40; 1940, 0.40; 1941, 0.48; 1942, 0.48; 1943, 0.52.
[3] Yearly applications of nitrogen (pounds per tree) were half the amounts applied in the high series.

TABLE 8.—*Relation of nitrogen content of foliage and fruit color of Rome Beauty apple trees to season of nitrogen application, 1939–43*

Relative rate of nitrogen application and treatment No. and description	Average nitrogen content of foliage [1]	Average color	Relative rate of nitrogen application and treatment No. and description	Average nitrogen content of foliage [1]	Average color
High: [2]	*Percent*	*Percent*	Low: [3]	*Percent*	*Percent*
1. Early-spring	1.87	56	7. Early-spring	1.69	66
2. Late-spring	2.04	43	8. Late-fall	1.73	63
3. Midsummer	1.91	39	9. Split (half late-spring, half early-fall)	1.75	56
4. Early-fall	1.89	52			
5. Late-fall	1.85	52	Difference necessary for significance at 5-percent point	.09	6.6
6. Split (half late-spring, half early-fall)	1.97	47			

[1] Leaves from terminal growth taken for analysis in late July or early August.
[2] Yearly applications of actual nitrogen (pounds per tree) were as follows: 1939, 0.40; 1940, 0.40; 1941, 0.48; 1942, 0.48; 1943, 0.53.
[3] Yearly applications of nitrogen (pounds per tree) were half the amounts applied in the high series.

Furthermore, the highest fruit color occurred in treatments with low-nitrogen levels and low rates of growth (treatments 7 and 8). This relation of nitrogen content of foliage to color development in the fruit is shown graphically in figure 12. Reference to this chart shows

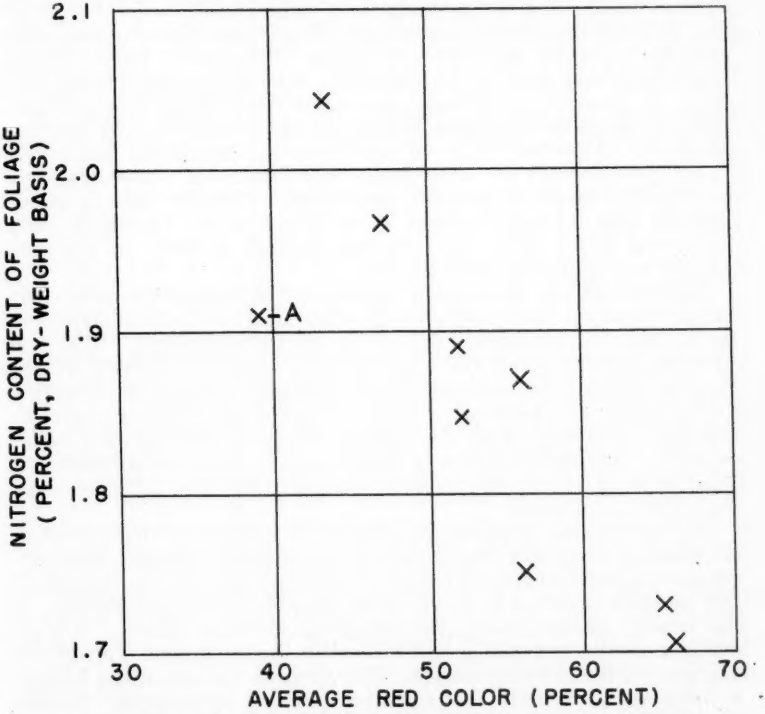

FIGURE 12.—Relation of average nitrogen content of foliage and average percentage of red color of fruit of Rome Beauty apples on young trees. x–A, Value for midsummer application.

approximately the same negative relation between nitrogen content of foliage and development of fruit color that was previously reported by the authors (*12*). With the midsummer treatment there was a much lower color of fruit than might have been expected from the nitrogen content of the foliage (fig. 12, x–A). An explanation of this variation is the fact that the yearly applications of nitrogen in this treatment were usually made at the time of the sampling of foliage for nitrogen analysis or immediately afterward. These young trees apparently absorbed and translocated nitrogen rapidly, and the nitrogen content of the foliage at the time of fruit coloring was relatively higher than at the sampling date. Thus, color development was poorer than would be expected from the nitrogen content of the foliage sampled (late July or early August).

DISCUSSION

In these studies nitrogen applications to mature apple trees made at five different times during the year, from early spring to late fall, failed to result in any essential differences in tree performance over a period of 4 years as measured by set, color, and yield of fruit and by general vigor of tree. The relation of nitrogen content of various tissues to tree vigor has been shown previously (*3*, *15*, *17*) and is rather generally recognized. Throughout these experiments extensive nitrogen analyses were made of blossoms, shoots, leaves, bark, wood, and roots of trees receiving the nitrogen at different seasons. In general, the nitrogen content of these tissues does not indicate any large or consistent differences due to time of nitrogen application. At certain times of year, however, significant differences in nitrogen content of some tissues were observed; these seemed to be related to season of nitrogen application. Thus, the data in table 1 indicate that nitrogen content of the roots in late March or early April was lower when nitrogen was applied in early spring than when it was applied at other seasons.

The root samples were taken just before the nitrate was applied in the early-spring treatment. In all other treatments the nitrate content of the soil was higher during the preceding months. This fact probably accounts for the slightly lower nitrogen content of the roots at the spring sampling. The nitrogen content of blossoms, young shoots, and leaves of trees receiving the early-spring treatment compared favorably with the content following treatments at other seasons. This indicates that in these vigorous trees, the nitrogen applied in early spring was in part taken into the tree within a short time after application.

The midsummer applications resulted in a relatively high level of nitrogen in roots and shoots during the dormant season. This application was made after the season's terminal growth was completed and could be expected to result in an increase in nitrogen content of the various tissues during the following dormant season. Nitrate nitrogen in the soil was at a low level in this treatment during the first part of the growing season. The nitrogen content of the foliage in June was lower than that for other times of application. In late summer, however, the nitrogen content of foliage was substantially the same for all application dates.

The examples just given serve to illustrate certain apparent trends

in nitrogen content of tissues as related to season of application. However, when the data as a whole are considered, it is rather obvious that under the conditions of these experiments different seasons of nitrogen application over a period of 4 years failed to influence materially the nitrogen level of mature apple trees. These results strongly suggest that, if the level of nitrogen metabolism is satisfactorily maintained within the tree, it makes little or no difference at what season of the year the soil supply is replenished.

These results were obtained with trees that had already received yearly applications of nitrogen prior to the initiation of the experiments. The rate of nitrogen application during the experiments was slightly in excess of the usual commercial rate for such trees. During the 4 years of the experiment the check trees, with no added nitrogen, yielded almost as well as the fertilized trees, although all their tissues showed a lower level of nitrogen and shoot growth was reduced. Fruit color on these trees was very much better than on those receiving nitrogen fertilizer. The soil in which the trees were growing is inherently fertile. A considerable amount of organic debris from old sod and fallen leaves had accumulated under the trees, and the check trees undoubtedly obtained considerable nitrogen from these sources. The superior quality of the fruit produced on these trees emphasizes the need for a critical evaluation of the effect of different levels of nitrogen on yield and quality of fruit on mature trees. The level of nitrogen appears much more important than the time of application in determining tree response.

Results of these studies with mature apple trees are at variance with those of a number of workers who reported considerable differences in tree response to season of nitrogen application (*1*, *11*, *15*, *16*, *17*). As pointed out earlier, in most instances such differences were obtained for a short period after applications of nitrogen to trees in moderate vigor or in most cases in extremely low vigor.

Results with young trees in these studies showed a very close relation between nitrogen content of foliage and growth of tree. In certain seasons of application the results with young trees are in close agreement with those obtained with mature trees. Thus, no significant difference was obtained in nitrogen content of foliage or in growth, when nitrogen was applied in early spring, early fall, or late fall. In contrast with the results with mature trees, however, the late-spring and midsummer applications resulted in a higher nitrogen level in the foliage, which in turn was associated with greater growth. Because of the variable production behavior associated with young trees for the first few years of bearing, little significance could be attached to the production performance of these trees during the course of these studies. It is reasonable to expect, however, that differences in growth rate would ultimately be reflected in fruit production.

The color records on Rome Beauty indicated a very close association between nitrogen content of the foliage, particularly in late summer, and fruit color. Crops of the Starking Delicious and York Imperial in this young orchard were too light and variable to make color records significant.

Sod, which might compete with the tree for applied nitrates, was removed prior to the start of the experiments. Results on the nitrate movement in the soil suggest possible advantage in applying nitrogen in the fall for trees growing in sod. When the nitrate was applied

in the fall most of it was in the second and third feet of soil the following spring, which would be below the dense mass of grass roots in the first foot of soil. Most of the nitrate applied in the spring remained in the top foot throughout the summer or until absorbed by the tree. These results indicate that trees growing in sod probably will obtain more of the nitrogen if it is applied in the fall. Experimental data bearing specifically on this point were not obtained.

The results on nitrate disappearance also emphasize that even the most available forms of nitrogen are taken up rather slowly by trees and that some absorption probably occurs throughout the year. Approximately half of the total nitrate applied in early spring was still recoverable in soil samples taken in late July, and approximately one-fourth could be recovered at the end of the growing season. The same general situation held with other times of application. Thus, enough nitrogen may be absorbed within a relatively short time to affect foliage color, but a relatively long period is required before most of the applied nitrogen is taken up by the tree. Maximum rate of absorption of nitrates by the trees was in early spring during the period of expansion of the leaf system, although rapid uptake occurred in midsummer if the trees were not previously well supplied with nitrogen.

CONCLUSIONS AND SUMMARY

Investigations were conducted through 4 years to determine the relation of season of nitrogen application to response in mature apple trees. Under the conditions of these experiments the following conclusions are indicated:

(1) On relatively old York Imperial and Delicious apple trees fruit set, color, and yield were not influenced by season of nitrogen application.

(2) Greater downward leaching of nitrate occurred when sodium nitrate was applied to the soil in early or late fall than when applied in spring or midsummer. With mature trees this greater leaching into the second and the third foot of soil apparently was not reflected in less nitrogen intake and tree growth.

(3) Nitrogen content of blossoms, shoots, leaves, bark, wood, and roots, which was determined throughout these experiments, did not indicate any large or consistent differences due to time of nitrogen application.

(4) After 4 years of treatment of mature apple trees no difference was found between fall and spring applications in the nitrogen reserve contained in the various tissues of the tree.

(5) Results with mature apple trees strongly suggest that, if the level of nitrogen metabolism is satisfactorily maintained within the tree, it makes little difference at what season of the year the soil supply is replenished.

Tests were conducted during 5 years with young Starking Delicious, Rome Beauty, and York Imperial trees. Late-spring and midsummer applications of nitrate nitrogen resulted in a higher nitrogen level in the foliage, which in turn was associated with greater growth and in Rome Beauty with less fruit color. No significant differences were found in nitrogen content of foliage, in growth, or in color of fruit when nitrogen was applied in early spring, early fall, or late fall.

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INHERITANCE OF RESISTANCE TO BACTERIAL WILT IN TOBACCO[1]

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INTRODUCTION

The occurrence of genetic resistance to bacterial (Granville) wilt, caused by *Bacterium solanacearum* E. F. Sm., in tobacco (*Nicotiana tabacum* L.) has been reported.[2] Different levels of resistance have been found in certain genotypes: Low resistance in numerous strains including certain flue-cured varieties, moderate resistance in T. I.[3] 79A[4] and Turkish Xanthi, and high resistance in 79–X and T. I. 448A.[5] The last-named genotype had no objectionable growth or quality characteristics. In later work Oxford 26,[6] a flue-cured variety with high wilt resistance and quality, was developed by selection from a cross of T. I. 448A × 400. Adequate data have been accumulated from the breeding work to permit a detailed report on the inheritance of wilt resistance. Such information should be of aid in solving the problem of combining resistance to wilt with resistance to other diseases and in coping with other problems arising from the development and use of wilt-resistant varieties.

MATERIAL AND METHODS

F_1 generations were grown for seed increase in the greenhouse during the winter. Seedlings of the F_2 and later generations were grown in steam-sterilized beds and transplanted into naturally contaminated soil in the field. Fertilization and cultural methods were similar to those used for flue-cured tobacco. Sites for test plantings were selected on the basis of uniformity trials in which 95 percent or more of known-susceptible varieties were killed by wilt. Single-row plots (fig. 1) of 25 to 150 plants were grown in completely randomized blocks, grouped according to generation or parentage so that closely related strains occurred in the same section of the field. The number

[1] Received for publication April 24, 1947. Cooperative investigations of the Division of Tobacco, Medicinal, and Special Crops, the North Carolina Agricultural Experiment Station, and the North Carolina Department of Agriculture.

[2] CLAYTON, E. E., and SMITH, T. E. RESISTANCE OF TOBACCO TO BACTERIAL WILT (BACTERIUM SOLANACEARUM). Jour. Agr. Res. 65: 547–554, illus. 1942.

[3] T. I. refers to accession number of Division of Tobacco, Medicinal, and Special Crops.

[4] A selection from a collection from Java.

[5] A selection from a collection from Colombia.

[6] SMITH, T. E., CLAYTON, E. E., and MOSS, E. G. FLUE-CURED TOBACCO RESISTANT TO BACTERIAL (GRANVILLE) WILT. U. S. Dept. Agr. Cir. 727, 7 pp., illus. 1945.

FIGURE 1.—*A*, Strain DSPA, selected from hybrids of Davis Special × Pinkney Arthur; *B*, wilt-susceptible variety; *C*, F_5 of (T. I. 448A × 400) × 401.

of replications ranged from 3 to 5 in most experiments. The amount of wilt was determined 12 or more weeks after transplanting, and was recorded as a disease index in which 0 equaled no symptoms and 100 the killing of all plants. The disease index was a weighted average of a uniform number of plants from each replication graded into 4 groups with assigned values of 0, 25, 50, and 100.

UTILIZATION OF THE LOW WILT RESISTANCE FOUND IN CERTAIN FLUE-CURED VARIETIES

Low resistance to wilt was reported by Garner, Wolf, and Moss.[7] Low resistance has also been found in numerous introduced genotypes [8] and in the flue-cured varieties Davis Special, Pinkney Arthur, and 400. Efforts to increase this resistance by line selection failed, but some success resulted from selection within intervarietal hybrids. Under conditions such that highly susceptible varieties usually had a disease index of 95 or more, Davis Special and Pinkney Arthur had indices that ranged from 74.7 to 82.0 (table 1). The F_1 of Davis Special × Pinkney Arthur was completely susceptible, but resistant plants occurred in the F_2. These were selfed, and there were established a number of F_3 lines that had significantly less wilt than either parent (table 1). After selection through the F_5, a composite F_6 (fig. 1) had a disease index of 55.8, as compared with 98.6 for a susceptible variety. This material was designated DSPA. In trial plantings on farmers' fields it had enough resistance to mature a full

[7] GARNER, W. W., WOLF, F. A., and MOSS, E. G. THE CONTROL OF TOBACCO WILT IN THE FLUE-CURED DISTRICT. U. S. Dept. Agr. Bul. 562, 20 pp., illus. 1917.

[8] See footnote 2, p. 27.

crop under conditions of moderate wilt severity. However, on the heavily infected experimental plots the DSPA strain showed much less resistance than T. I. 448A, which generally had a disease index of approximately 10.

TABLE 1.—*Wilt indices of Davis Special and Pinkney Arthur tobaccos and of the hybrids and selections, 1941–43*

[0=No symptoms of wilt and 100=killing of all plants]

Genotype	Disease index of indicated generation			
	F_1	F_2	F_3	F_4
Davis Special parent			[1] 80.7	
Pinkney Arthur parent			[1] 74.7	[1] 82.0
Davis Special × Pinkney Arthur	96.7	90.0		
Pinkney Arthur × Davis Special		94.0		
Selection 1			22.6	34.5
Selection 2			29.0	39.5
Selection 3			32.0	40.5
Selection 4			32.6	67.0
Selection 5			34.3	73.0
Selection 6			53.3	79.5
Selection 7			57.6	79.5
Selection 8			58.3	
Selection 9			60.7	
Selection 10			66.3	
Selection 11			67.0	
Least significant difference (P=0.01)			30.5	25.7

[1] From plantings of the regular variety indicated.

ATTEMPTS TO DEVELOP AN IMMUNE LINE

The additive nature of wilt resistance occurring in strains with moderate resistance was shown by selection of 79–X from the cross T. I. 79A × Turkish Xanthi.[9] An effort was made to find whether the high resistance in T. I. 448A and 79–X could be accumulated to produce immunity. In a cross of moderately resistant strain × highly resistant strain (DSPA and a hybrid having the full resistance of T. I. 448A, table 1), it was not possible to recover more resistance than that of T. I. 448A by selection in the F_2 and F_3. The cross between 2 highly resistant strains was of the most interest. The strains 79–X and T. I. 448A had disease indices of approximately 10, and a distinct reduction of this value would have approached immunity. The F_1 population from T. I. 448A × 79–X had significantly more wilt than either parent, indicating that the 2 strains had different genes for resistance. A total of 117 F_2 plants that appeared free from wilt were tested as F_3 lines in 1941. Of these, 13 had a lower disease index than T. I. 448A. Healthy plants were selected from the most resistant lines, and this procedure was continued through the F_6. The final results were negative, as strains with more resistance than T. I. 448A were not recovered. The reason for the failure may have been that critical wilt severity did not occur regularly enough for effective selection within the disease-index range 10 to 0.

[9] See footnote 2, p. 27.

INHERITANCE OF RESISTANCE IN CROSSES OF T. I. 448A WITH FLUE-CURED VARIETIES

T. I. 448A was crossed with several flue-cured varieties. Data were obtained on the inheritance of resistance in 8 generations of the original cross (table 2). The F_1 was almost as susceptible as the susceptible parents, with disease indices of 90.4 and 94.7, respectively. Thus resistance was recessive. The F_2 had less wilt than the F_1 or the susceptible parents. Individual F_2 plants were selected for resistance; of 166 such selections tested as F_3 lines, 7 were highly resistant. The other 159 gave a distribution all the way to complete susceptibility. Selection in the F_3 and F_4 generations was required to eliminate all susceptible lines. Continued selection in the F_5 through the F_7 maintained resistance equal to that of T. I. 448A but did not increase it.

TABLE 2.—*Wilt indices of hybrids of resistant T. I. 448A × susceptible varieties of tobacco, 1941–43*

[0=No symptoms of wilt and 100=killing of all plants]

Genotype	Total tests	Tests with disease index [1] of—										Average disease index
		1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	
Resistant parent (T. I. 448A)	12	3	9	0	0	0	0	0	0	0	0	12.5
Susceptible parents	23	0	0	0	0	0	0	0	0	2	21	94.7
F_1	5	0	0	0	1	0	0	0	0	3	2	90.4
F_2	7	0	0	0	0	0	2	1	1	3	0	71.8
F_3	166	7	22	23	26	28	18	23	11	7	1	42.8
F_4	42	11	9	7	7	4	4	0	0	0	0	23.8
F_5	11	3	6	2	0	0	0	0	0	0	0	13.7
F_6	23	23	0	0	0	0	0	0	0	0	0	3.1
F_7	4	3	1	0	0	0	0	0	0	0	0	10.5
F_8	8	3	4	1	0	0	0	0	0	0	0	15.6
Resistant F_3 × susceptible parent:												
F_2	13	0	0	0	0	0	5	2	2	2	2	70.6
F_3	169	1	8	15	15	28	31	29	21	15	6	56.8
F_4	67	28	18	17	2	1	1	0	0	0	0	15.2
F_5	38	11	14	12	1	0	0	0	0	0	0	17.0
F_6	17	2	13	2	0	0	0	0	0	0	0	15.8

[1] Each test was a replicated planting of 75 to 300 individuals. Over 52,000 plants are represented in the entire table.

Resistant F_3 selections were backcrossed to susceptible varieties, and data on the F_2 through the F_6 are also summarized in table 2. The pattern was similar to that for the results obtained from the original cross. Successive generations of the original cross and the first backcross had disease indices, respectively, as follows: F_2, 71.8 and 70.6; F_3, 42.8 and 56.8; F_4, 23.8 and 15.2; F_5, 13.7 and 17.0; F_6, 3.1 and 15.8. The results showed that the full resistance of T. I. 448A was recovered from the original and the first backcross even though resistance was controlled by a number of genes. Inheritance of resistance in hybrids of 79–X × flue-cured varieties followed the same pattern in less extensive data.

RELATION OF SUSCEPTIBLE PARENT TO THE RECOVERY OF RESISTANCE

Attention was also given to the effect of different flue-cured varieties on the recovery of resistance in the hybrids. Data on the F_1, F_2, and F_3 of hybrid families arising from crosses of T. I. 448A with 6 flue-cured varieties are shown in table 3. All F_1 populations were highly susceptible. The F_2 populations from crosses with Davis Special and 400 had significantly less wilt. In the F_3, the Davis Special family had few resistant selections but the 400 family had many resistant selections. Of 55 F_3 lines of 400 parentage, 19 had a disease index of 20 or less and hence were highly resistant. In table 3, 141 F_3 lines are reported; very high resistance (disease index of 10 or less) occurred in 7 of these; of these 7, 5 came from the cross with 400. The superior combining ability of this variety, which resulted in numerous segregates with wilt resistance, was a major factor in the development of a commercial flue-cured tobacco with high resistance to wilt.

TABLE 3.—*Effect of crossing T. I. 448A with susceptible varieties of tobacco on recovery of resistance to wilt, 1941–43*

[0=No symptoms of wilt and 100=killing of all plants]

Susceptible variety	Average disease index of—		F_3 lines [1] recovered with indicated disease index									
	F_1	F_2	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100
			Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Gold Dollar	98.9	82.0	1	5	5	5	8	3	3	2	1	1
400	89.7	60.0	5	14	14	9	4	3	5	1	0	0
Davis Special	------	58.5	0	1	0	4	6	8	9	2	4	0
Cash	85.3	82.0	1	0	1	1	3	2	0	1	0	0
Bonanza	87.3	79.5	0	0	0	3	1	1	1	0	0	0
Virginia Bright Leaf	91.0	81.5	0	0	0	1	1	0	0	0	1	0
Least significant difference (P=0.01)	[2]	11.2	------	------	------	------	------	------	------	------	------	------

[1] Each line was a planting replicated 3 times from a selfed F_2 selection.
[2] Not significant.

DISCUSSION

Wilt resistance from three separate sources has been investigated with respect to degree and inheritance. Certain flue-cured varieties, Davis Special, Pinkney Arthur, and 400, had low resistance. A cross between two of these produced, after selection through the F_5, a genotype with a disease index of about 55 as compared with 75 to about 80 for the parents and 95 to 100 for susceptible varieties. Some years ago obtaining this degree of resistance would have been regarded as a major achievement. At present the higher resistance obtained in foreign collections is preferred. The best resistant genotypes obtained were 79–X, which was developed from the cross of moderately resistant T. I. 79A with Turkish Xanthi, and T. I. 448A. Both gave disease-index values of about 10. When 79–X was used as the source of resistance, the resistant segregates tended to have small leaves of poor quality. T. I. 448A, on the other hand, produced highly resistant lines with good agronomic characters. The resistance in each case was recessive and controlled by multiple genes. Resistance in

T. I. 448A and that in 79–X were not identical, because the F_1 of a cross of these two was more susceptible than either parent. An effort was made to develop an immune genotype by selection in the hybrids of T. I. 448A × 79–X. The attempt failed, presumably because it was not possible to select effectively within a disease-index range of 10 to 0. Therefore, resistant genotypes exist as (1) T. I. 448A, (2) 79–X, selected from a cross of T. I. 79A × Turkish Xanthi, and (3) DSPA, selected from a cross of Davis Special × Pinkney Arthur. T. I. 448A was the resistant breeding stock in the development of Oxford 26. The other genotypes constitute a reserve of possible value should physiologic specialization occur in the pathogen.

Oxford 26 was released for increase of seed stocks 5 years after T. I. 448A was crossed with flue-cured varieties. Factors that contributed to its rapid development were (1) lack of objectionable quality or growth characters in T. I. 448A, (2) recessive resistance, (3) occurrence of increased numbers of resistant segregates in crosses with 400, and (4) growth of large populations under such severe disease conditions that the few highly resistant segregates could be picked out in each generation. Over 52,000 plants were grown in the F_2 through the F_6 generations of the original and first backcrosses of T. I. 448A × flue-cured varieties; 532 individual-plant selections were tested. Only 1 selection was of sufficient value to be released as a variety, but 5 genotypes had potential value. It is significant that all 5 of the potentially valuable selections were of 400 parentage. Hence, rapid progress was possible because of the combining performance of 400, which assisted in the recovery of resistance, and because of the growth of large populations to facilitate recovery of quality and growth characters. Resistance to other tobacco diseases is controlled by multiple genes. The results of these studies on wilt resistance may suggest methods of obtaining rapid progress with other genetic material of this type.

SUMMARY

Resistance to bacterial wilt was recessive and controlled by multiple genes. The flue-cured varieties Davis Special and Pinkney Arthur were slightly resistant. It was possible to select a moderately resistant genotype from a cross of the two. Genotypes possessing high resistance were T. I. 448A and 79–X. Segregates from crosses of T. I. 448A × flue-cured varieties were much superior in growth and quality characters to segregates from similar crosses in which 79–X was the source of resistance. Of the flue-cured varieties used as parents, variety 400 produced the most progeny with high resistance and good agronomic characters. After crossing T. I. 448A with a susceptible tobacco it was necessary to continue selection to the F_5 generation before the full resistance was recovered. This resistance was not increased beyond the T. I. 448A level by selection through the F_7 generation. After backcrossing resistant segregates to susceptible tobacco the full T. I. 448A resistance was again recovered in the F_5 generation. Because of high resistance, yield, and quality standards, rapid progress was possible only through the use of large populations and conditions permitting critical evaluation. Only 5 potentially valuable genotypes have been retained from 52,000 hybrid plants of T. I. 448A parentage.

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